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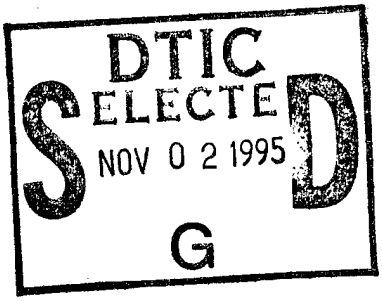

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FOREWORD

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10th Annual Conference on Human Retroviral Testing

March 1995
Reno, Nevada

CONFERENCE PURPOSE

A forum on national and international laboratory-related retroviral issues which will allow for an exchange of information and ideas and encourage discussion of current issues.

DESIRED CONFERENCE OUTCOMES

1. To review state-of-the-art technologies and produce summary papers on current retrovirus issues.

2. To identify specific issues that require follow-up and establish action plans.

3. To raise questions even if the answers are not known.

Association of State and Territorial
Public Health Laboratory Directors

ASTPHLD

The Association of State and Territorial Public Health Laboratory Directors (ASTPHLD) represents state and territorial public health laboratory directors throughout the United States. The Association maintains a Headquarters Office and seven Area Resource Offices, and operates a National Laboratory Training Network that forms alliances among federal, state and local health agencies and private sector organizations to develop and promote the delivery of localized laboratory training programs based on documented need. The Association also organizes and presents scientific conferences and symposia relevant to the testing activities of public health laboratories. ASTPHLD operates exclusively as a scientific and educational organization.

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William J. Hausler, Jr., Ph.D., Iowa

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Roger Dodd, Ph.D., American Red Cross

Jane Getchell, Dr.P.H., Iowa

Paul Mied, Ph.D., Food and Drug Administration

Charles Schable, M.S., Centers for Disease Control and Prevention

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Centers for Disease Control and Prevention

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Memorial Blood Center of Minneapolis
Minneapolis, Minnesota

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CONFERENCE SUMMARY

The Tenth Annual Conference on Human Retrovirus Testing sponsored by the Association of State and Territorial Public Health Laboratory Directors (ASTPHLD) was held March 6 to 9, 1995, in Reno, Nevada. The conference, which was attended by more than 300 representatives of public and private sector laboratories as well as test kit manufactureres, emphasized three themes: new HIV variants, international issues and ASTPHLD involvement, and testing of newborns.

New HIV Variants and Their Effects on HIV Testing

HIV variability was expertly discussed from both the European and U.S. points of view. Guido van der Groen (*Chairman, Department of Infection and Immunity, Adjunct Chief Microbiology Division, World Health Organization (WHO) Center on AIDS, Antwerp, Belgium*) summarized information on HIV-1, type O viruses presented just days earlier at a world Health Organization meeting. Sequence data for 21 type O isolates are now available. The immunodominant region of gp41 in the type O isolates varies fromt that in the main group of HIV-1 isolates -- this is probably why sera from some patients were non-reactive to envelope antigens when tested on conventional Western blots. J. Richard George, Dr.P.H. (*Chief, Developmental Technology Section, Division of HIV / AIDS, Centers for Disease Control and Prevention, Atlanta, Georgia)* complemented this talk by describing CDC's surveillance for unusual variants of HIV. International collaborations to look for HIV variants were established in 1994 between CDC and Uganda, Kenya, Côte d' Ivoire, and Brazil. Dr. George pointed out that nearly all HIV diagnostic kits have been made with viral lysates of a subtype B (III_B) virus or gene products derived from its sequence.

Both speakers agreed that there are HIV-infected persons whose sera do not react with the gp41 III_B epitopes, but the number of such persons is unknown. Retrospective studies in the United States have detected no unusual variants, and prospective studies to address this issue are beginning. More sera are needed from patients infected with HIV variants, and current assays need to be assessed against new information on variation.

Arthur Brown, M.D., LTC, M.P.H. (*Chief, Diagnostic Retrovirology, Walter Reed Army Institute of Research, Rockville, Maryland)* presented data on changing virologic face of the HIV epidemic in the United States and Europe. In U.S. military personnel who seroconverted and in patients with acute retroviral syndrome in Geneva, the frequency of zidovudine (AZT)-resistant virus has increased from zero to approximately 10% in the early 1990s. Similar frequencies are found in civilians in the United States and Australia.

HIV Testing of Newborns

A half-day forum with four speakers addressed issues of HIV testing and the newborn. The impetus for this session was the recent report of decreased maternal-neonatal transmission due to AZT prophylaxis. Yvonne Maldanado, M.D. (*Division of Pediatric Infectious Disease, Stanford University School of Medicine, Stanford California)* reviewed the results of this study, which was discontinued because the transmission rate was significantly lower in mother-child pairs receiving AZT than among those receiving placebo (8% vs. 25%). Dr. Maldonado pointed out that the time of infection of a neonate is usually not known; an estimated two-thirds of infected neonates are infected perinatally, and an additional 10% to 30% may be infected in association with breast-feeding. Because of the high death rates associated with pneumocystis pneunomia in the first year of life, diagnosis in the first weeks to months

after birth is essential for timely prophylaxis.

The national anonymous survey of HIV prevalence in newborns was reviewed by Marta Gwinn, M.D. (*Chief, Clinic and Special Surveys Section, Division of HIV / AIDS, Centers for Disease Control and Prevention, Atlanta, Georgia).* This survey represents one of the few HIV surveillance systems in the United States that describe prevalence of infection rather than prevalence of AIDS. Results from 1993 show that approximately 7,000 HIV-infected, pregnant women delivered an estimated 1,000 to 2,000 infected children.

While some argue that this survey should be unblinded, CDC recommends maintaining it as is for its unique epidemiologic value and to provide input for policy decisions; CDC also recommends offering HIV testing to all pregnant women. Dr. Gwinn used survey data to show that the number of women tested to detect one HIV-infected woman would be from <200 to 5,000 (national average, 500 to 600), depending on the prevalence of HIV in a given state.

Diagnosis of HIV in newborns was reviewed by I. Celine Hanson, M.D. (*Associate Professor of Pediatrics, Baylor College of Medicine, Texas Children's Hospital, Houston, Texas).* Three points were emphasized: No clinical signs distinguish HIV-infected children; risk for infection among exposed children appears to vary directly with maternal viremia (p24 Ag; quantitative RNA is not yet available) and inversely with CD4 count; and diagnosis in the first months of life depends on virologic assessment (polyerase chair reaction (PCR), culture, (p24 Ag), and these assays may approach 95% sensitivity when the child is 2 to 3 months old. Dr. Hanson said that confirming noninfection is now more difficult than diagnosing infection.

For the first time in the 10-year history of the conference, ethical issues were discussed formally. Ronald Bayer, Ph.D. (*Professor, Columbia University School of Public Health, New York, New York)* reviewed the ethical considerations of perinatal screening. He pointed out that although maternal and neonatal screening raised many similar issues, the ethical questions differed, and the main argument against mandatory

testing of pregnant women was that a woman could not be forced to take AZT and, thus, testing itself was not directly linked to decreased infection rates in the newborn.

International Issues and Involvement

An international perspective on HIV testing was brought to the Conference by presentations that focused on India and Latin America. Mahadeo P. Verma, Ph.D. (*Director, Delaware Public Health Laboratory, Division of Public Health, Smyrna, Delaware)* described the results of a project funded by a 12-month study grant from the World AIDS Foundation to provide training on HIV testing to laboratories in India.

Four Indian facilitators were trained in the United States and the provided translation and other assistance to eight ASTPHLD faculty who gave 6-day workshops in four training centers in India. Training focused on enzyme immunoassays (EIAs), linking trainees with staff from Indian reference centers, and establishing training materials and trainers for future workshops to be conducted by Indian staff.

Laboratory aspects of HIV testing in Latin America and the Caribbean were discussed by José Ramiro Cruz, D.Sc. (*Regional Advisor on Laboratory and Blood Services, Pan American Health Association (PAHO))*, who described the spectrum of HIV incidence rates and testing algorithms. PAHO is asking regional countries to assess their algorithms in terms of sensitivity / specificity and cost. PAHO aims to support national laboratories by providing guidelines and quality assurace; proficiency testing is encouraged and is provided by CDC (Centers for Disease Control and Prevention). Dr. Cruz invited ASTPHLD to work with PAHO in achieving those goals.

Additional Topics

Prevention of opportunistic infections in patients with AIDS was reviewed by

Jonathan Kaplan, Ph.D. (*Assistant to the Director for Infectious Diseases, Division of HIV / AIDS, Centers for Disease Control and Prevention, Atlanta, Georgia).* For 18 microorganisms that cause opportunistic infections, recommendations have been formulated for preventing infection, treating disease, and preventing relapse. CD will publish these in the summer of 1995. Dr. Kaplan emphasized that preventing opportunistic infections in AIDS patients depends on diagnosis of HIV infection and, therefore, on HIV testing.

The issues involved in the use of the external controls were reviewed by Margaret Hanson, Ph.D. (*Virology Laboratory Manager, Memorial Blood Center of Minneapolis, Minneapolis, Minnesota),* who used EIAs to illustrate the main points of the discussion. It was suggested that low-positive external controls are the most useful, especially in monitoring test system performance. When using external controls, it is necessary to specifically define the purpose and frequency of their use and to target values and corrective actions to be taken.

Paul Mied, Ph.D. (*Acting Director, Division of Transfusion Transmitted Diseases, CBER, Food and Drug Administration, Rockville, Maryland),* summarized the results of an FDA-sponsored meeting held 6 months previously which focused on detecting HIV infection through genetic-based technologies during the preseroconversion window. It was estimated that current third generation EIAs have narrowed this window to 3 to 4 weeks. Genetic technologies theoretically close this window further, but not completely.

One conclusion of the FDA meeting was that none of the technologies were ready for use in the volumes of specimens and through-put required by blood banks and that the cost per potential infection detected was prohibitive.

The basics of HIV testing were reviewed both in a session conducted by Charles Schable, M.S. (*Chief, Serology Section, Division of HIV / AIDS, Centers for Disease Control and Prevention Atlanta, Georgia),* and a Western blot workshop organized by

Judith Wethers, M.S. (*Director of Testing, Retrovirology Laboratory, New York State Department of Health, Albany, New York).* The continued interest in these presentations is derived from the turnover of technical personnel in HIV testing laboratories and the continued need for test improvement, especially at the confirmatory level.

Additional sessions discussed testing of alternative specimens (oral fluids, dried blood spots), home collection, CD4 cell quantitation, PCR/LCR, and p24 Ag after immune complex dissociation. Women's health issues and CDC participation in this arena were discussed by Wanda K. Jones, Dr.P.H. (*Acting Associate Director for Women's Health, Centers for Disease Control and Prevention, Atlanta, Georgia).* The founder of the ASTPHLD Conference, William J. Hausler, Jr., Ph.D. *(Director, State Hygienic Laboratory, University of Iowa, Iowa City, Iowa)*, critiqued its first decade, and David Carpenter, Ph.D. (*Director, Division of Laboratories, Illinois Department of Health, Springfield, Illinois)*, president, ASTPHLD, discussed its recent evolution and growth. The 11th ASTPHLD Conference will be held in Orlando, Florida, in March 1996.

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3 **Variable Hematology Results Due to Specimen Alterations Encountered In Developing a Protocol for a T-Lymphocyte Immunophenotyping Proficiency Testing Program**
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ABSTRACT AUTHORS

POSTER NUMBER 1

PERFORMANCE EVALUATION OF THE FACSCOUNT™ SYSTEM FOR ABSOLUTE CD4 COUNTS LESS THAN 50 CELLS/µL

Shiba A*, Ward DM*, Lock M*, Webster K*.
*Becton Dickinson Immunocytometry Systems • San Jose, CA.

The FACSCount System is a complete system for the enumeration of absolute $CD4^+$, $CD8^+$, and $CD3^+$ T lymphocytes in whole blood. The single instrument platform with dedicated software and reagents is easy to use and designed to improve reproducibility. In previous evaluations, the FACSCount System was shown to be equivalent to the standard methodology of a FACScan™ flow cytometer and hematology analyzer. This evaluation focuses on the performance of the FACSCount System when enumerating absolute CD4 counts less than 50 cells/µL. Data presented in this poster compares the accuracy and precision of the FACSCount System for determination of absolute CD4 lymphocyte counts of less than 50 cells/µL.

Evaluation of system accuracy was determined by comparison of whole blood samples analyzed in parallel with the FACSCount System and the standard methodology. The standard methodology consisted of a FACScan flow cytometer and a hematology analyzer. FACSCount System reproducibility was estimated from samples stained with four replicates each.

Data comparing absolute counts from the FACSCount System with counts from standard methodology are presented. Estimates of the within patient, between stain precision for CD4 counts less than 20 , and for counts between 21 and 50 cells/µL are presented.

POSTER NUMBER 2

Flow Cytometric CD4 Cell Determination Based Upon CD3 Gating in HIV Infected Individuals

ALEXANDER, T., R. BALAJ and L. DEFINE, Summa Health System, Akron, OH 44309

CDC Guidelines for CD4 Immunophenotyping suggest a 6 tube, 2 color panel, but recommend evaluating 3-color approaches. Nicholson, et al., (Cytometry 14(6), 685-689, 1993) showed acceptable QC using CD45/SS gating and a single CD3/4/45 tube. Often CD4 and CD8 values are of interest and a single tube which could provide both values would be useful. Recently introduced software for the Ortho Cytoronabsolute and BD FACScount allow for CD4 determinations based upon CD3 gating. We tested the hypothesis that CD3/SS gating in a CD3/4/8 tube would provide results comparable to those obtained using either conventional light scatter gating or CD45/SS gating and provide both CD4 and CD8 values. 106 specimens obtained from HIV infected individuals were assayed for CD4% using a CD3/4/45 tube gated on FS/SS and CD45/SS, and a CD3/4/8 tube gated on CD3/SS. CD4 values were expressed as the % of CD3 positive cells for comparison. CD3/SS gating vs light scatter gating showed a linear regression equation of Y=0.99X+0.58 with an R2 of 0.99. CD45/SS gating vs FS/SS gating had a regression of 0.93X+0.25 with an R2 of 0.83. CD3/SS vs CD45/SS had an equation of 0.93+0.81 with an R2 of 0.82. CD3/SS gating was simpler to perform than CD45/SS gating due to the specificity of CD3 for lymphocyte populations and was reproducible (R2 = 0.98 for tube to tube variability). Our data confirms that CD3/SS gating is an appropriate method for determining CD4 values.

POSTER NUMBER 3

Variable Hematology Results Due to Specimen Alterations Encountered In Developing a Protocol for a T-Lymphocyte Immunophenotyping Proficiency Testing Program

CROSS, G.D.[1]; SCHALLA, W.O.[1]; TAYLOR, R.N.[1]; GAUNT, E.E.[2]; COHN, R.D.[2]; SEILKOP, S.K.[2]; HOLZWORTH, D.A.[2]
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Care and management of HIV-infected patients and the diagnosis of AIDS depend on accurate and reliable T-lymphocyte immunophenotyping (TLI) results. For developing a protocol for a national proficiency testing (PT) program, it was determined that a preliminary hematology study was necessary to provide information concerning the effects of specimen age and environmental factors on absolute lymphocyte counts. Specimens from 10 HIV-seropositive and 10 HIV-seronegative donors were subjected to two storage temperatures and two shipping conditions, and analyzed at 6-hour intervals. Three hematology instruments employing different techniques for counting and differentiating blood cells were used to evaluate identical specimens. Both the measured lymphocyte counts and their associated measurement variabilities changed over time. These changes were donor specific and dependent upon the particular hematology instrument and specimen storage temperature, e.g., measurement variability in aged specimens tended to be greater at 4°C than at ambient temperature. The effect due to different shipping conditions was comparatively minor and was only evident for certain instruments and storage temperatures. Measurement variability was homogeneous across donor specimens at 6 hours (baseline), but not at any time thereafter. Measurement variabilities were not explained by the HIV-antibody status or antiretroviral treatment of the donor.

POSTER NUMBER 4

Trends in Compliance of Laboratories Performing T-lymphocyte Immunophenotyping by Flow Cytometry with CD4-Cell Testing Guidelines.

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Information about the testing performance and the procedures used by laboratories in the CDC Model Performance Evaluation Program (MPEP) is obtained for laboratories that perform human T-lymphocyte immunophenotyping (TLI) by flow cytometry. The performance results as well as testing information are used to measure changes over time and to document adherence to guidelines. Results from the June 1992, October 1992, March 1993, September 1993, and the March 1994 shipment panels were compared with recommendations of the CDC *MMWR* CD4-Cell Testing Guidelines to estimate how well some guidelines were being followed and whether compliance had improved. The percentage of laboratories using the recommended monoclonal antibody panels steadily increased, with the percentages being 31.0% in June 1992, 39.9% in October 1992, 54.0% in March 1993, 56.0% in September 1993, and 60.2% in March 1994. There were 77 combinations of monoclonal antibodies used by the 277 laboratories reporting results, but 66% of the laboritories used one of five most common combinations and 73% used one of the 11 most common combinations. The percentage of laboratories using whole-cell lysis was relatively constant between 95.9% and 97.3% in these surveys, and the percentage of those that fixed their cells before flow cytometric analysis also was relatively constant between 95.9% and 97.3%. The number of laboratories reporting results ranged from 262 to 279 in these surveys.

POSTER NUMBER 5

DOES THE CURRENT LEVEL OF LABORATORY RELIABILITY OF CD4+ ABSOLUTE COUNTS MERIT THE CURRENT LEVEL OF PHYSICIAN CONFIDENCE IN THIS TEST?

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We estimated physician confidence in quality requirements for CD4+ testing from a nationwide survey of 498 responding physicians (a 33% response rate) specializing in infectious diseases, internists and family practitioners practicing in US metropolitan areas with high AIDS infection. Fifty-one percent found the accuracy of T-cell subsets very or completely satisfactory while only 7% rated it less than satisfactory. Physicians reported equal levels of confidence in CD4+ absolute counts and percentages with more than 72% expressing high or complete confidence in both measures. In general, there was no demand for major improvements in precision of CD4+ testing. Using a clinical scenario, when confronted with a significant drop in CD4+ count most physicians (53%) reported that they would reorder the test while 23% would initiate antiretroviral therapy.

We estimated the current level of within and between laboratory precision for CD4+ T-cell subsets using a series of blinded whole blood proficiency test specimens. Between laboratory precision of absolute counts ranged from 22% to 66% in contrast to a range of 8% to 15% in CD4+ percents. Unfortunately, physician impressions of precision are much more precise than empirical estimates from proficiency surveys.

Physicians using absolute counts should recognize the added potential for error introduced by the need for the total lymphocyte count to calculate the number of CD4+ cells. We recommend the use of percent counts wherever possible which are inherently less subject to laboratory analytic variations.

POSTER NUMBER 6

TRAx® CD8: Study Results From a Two Site Trial with HIV-positive Patients and Apparently Healthy Donors to Determine the Correlation to Flow Cytometry.

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TRAx® CD8 Test Kit is a simple ELISA which uses EDTA whole blood that has been lysed with a proprietary lysis reagent. The pretreatment of the specimen with the lysis reagent releases the CD8 protein from the cells. Specimens can then be assayed by EIA or stored at -20°C or -70°C for later study. The TRAx assay is performed in microtiter wells. Briefly, a monoclonal antibody to an epitope of the CD8 molecule is adsorbed onto polystyrene wells. An HRP-conjugated monoclonal antibody to a different epitope is added to the wells. The TRAx CD8 Kit Standards, lysed/diluted Kit Controls or lysed/diluted Samples are also added to the wells at this time, completing the sandwich. After a 3 hour incubation, the wells are washed to remove any unbound HRP-conjugate, and a substrate is added to the wells. The resultant color is directly proportional to the amount of CD8 protein in the wells. After a brief incubation the substrate reaction is stopped and the resulting color is read on a spectrophotometer at 450 nm. Unknown samples are read off of the Standard curve and reported as cells/uL.

Using the TRAx CD8 Test Kit a two site trial was run to establish the relationship between total CD8 protein measured by ELISA and the absolute CD8 T lymphocytes measured by flow cytometry and hematology. One site used for this study was in the northeast and the other was in the south. Both HIV-positive and normal donors were collected and processed at each site. In addition, each site did the flow cytometry/hematology analyses and ran the TRAx CD8 Test Kit. The correlation to flow was calculated using only the HIV-positive patients (98 subjects). The correlation to flow cytometry was determined to be 0.989. The data from this study were also used to calculate a conversion equation from units/mL to cells /uL for the TRAx CD8 Test Kit.

A third site, and the normal donors from the above sites, were used to validate this conversion equation. A total of 258 subjects were used to test this equation. The correlation to flow was determined to be 0.915. A small percentage of the HIV-positive samples were also diagnosed with Tuberculosis or Syphilis. No effect was observed due to these disease states or from the drugs associated with HIV therapy. In conclusion, the TRAx CD8 Test Kit offers a reliable and simple alternative to Flow Cytometry for the enumeration of CD8+ T cells. Clinical trial studies are currently underway to validate the conversion equation in additional HIV-positive and normal samples.

POSTER NUMBER 7

EVALUATION OF A MODIFIED HIV-1/HIV-2 RECOMBINANT/PEPTIDE BASED ANTIBODY SCREENING EIA

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Background: Specimens from individuals infected with HIV-1 Group O have been identified in clinical settings in Europe and in West Africa. These specimens exhibit discrepant reactivity with various antibody detection assays. The addition of an HIV-1 Immuno Dominant Region (IDR) peptide to the Abbott HIV-1/HIV-2 3rd Generation EIA improves detectability of these Group O specimens. The purpose of this investigation is to compare the overall performance of the peptide modified HIV-1/HIV-2 antibody assay to the Abbott HIV-1/HIV-2 3rd Generation EIA (test of record).

Method: In the European clinical evaluation, the following were tested in the aforementioned assay formats: 2 HIV-1 Group O positive samples, 3041 random blood donor specimens, 315 HIV-1 Western Blot (WB) positive samples 208 HIV-2 WB positive samples, and serial bleeds from 20 HIV-1 seroconverting individuals.

Results: The peptide modified assay detected 2/2 HIV-1 Group O positive samples which the test of record failed to detect. The initial reactive rate (IRR) and repeat reactive rate (RRR) for the improved assay were 6/3041 (0.20%) and 0/3041 (0.0%), respectively; for the test of record, the rates were 9/3041 (0.30%) and 0/3041 (0.0%). Both assays detected 315/315 HIV-1 WB positive samples and 208/208 HIV-2 WB positive specimens. Seroconversion sensitivity was comparable between the two assay formats; the peptide modified assay detected 98/150 bleeds and the test of record detected 97/150.

Conclusion: These data suggest that the peptide modified HIV-1/HIV-2 antibody assay will offer increased HIV-1 Group O sensitivity without compromising specificity, HIV-1 seroconversion or HIV-2 sensitivity.

POSTER NUMBER 8

COMPARISON OF A HIV-1/HIV-2 RECOMBINANT STRIP IMMUNOBLOT ASSAY AND HIV-1 WESTERN BLOT IN DETECTING HIV-1 "O" SUBTYPE SAMPLES

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Objective: To determine the sensitivity and specificity of Chiron® RIBA™ HIV-1/HIV-2 SIA compared to Cambridge Biotech HIV-1 Western blot (FDA licensed) in detecting HIV-1 subgroup "O" samples.

Methods: Eighteen samples from HIV-1 infected individuals which are characterized as subgroup "O" were tested by Cambridge Biotech HIV-1 Western blot and Chiron® RIBA™ HIV-1/HIV-2. Ten were tested at Institut Fournier and eight at Centers for Disease Control. The tests were performed according to manufacturers' instructions; the positive criteria definitions used were those recommended by the manufacturers'.

Results: 10/18 were positive and 8/18 were indeterminate by Western blot using ASTPHLD/CDC criteria. 6/8 Western blot indeterminate results were due to weak or absent gp160/gp120 and/or gp41 (env transmembrane tetrameric/trimeric and monomeric forms respectively) band reactivity; 2/8 Western blot indeterminates were due to weak or missing gp41 tetrameric (gp160) and p24 reactivity. All samples (18/18) were positive by RIBA™ HIV-1/HIV-2 SIA and were classified as HIV-1.

Conclusion: Chiron® RIBA™ HIV-1/HIV-2 SIA appears to be more sensitive than Western blot in detecting, as positive, HIV-1 subgroup "O" samples and is capable of discriminating these samples as HIV-1 rather than HIV-2. Western blot appears to have reduced sensitivity to envelope transmembrane antibodies in some samples.

POSTER NUMBER 9

Improved Specificity of a HIV -1/2 Recombinant Strip Immunoblot (SIA) Compared with HIV-1 Western Blot .

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Background: Non-specific p24 reactions in Western blot are not uncommon when confirming HIV-1 or HIV-1/2 ELISA repeat reactive samples from low risk populations. Confirmatory data, (positive, negative or indeterminate results) reflect both HIV-1/2 antibody prevalence in the tested population as well as false positive reactions produced by the screening ELISA. It has been demonstrated that combinant gag proteins in a immunoblot format tend not to react with Western Blot non-specific p24 reactive samples. The purpose of this study is to determine the specificity of a new HIV-1/2 recombinant strip immunoblot assay (SIA) when testing non-specific p24 only samples.

Design: 108 ELISA repeat reactive (but HIV infected) blood donor samples, having p24 only reactivity on HIV-1 Western blot, were tested by HIV-1/2 SIA. Samples were originally screened using 3 different ELISA tests; DuPont HIV-1 EIA, Abbot HIV-1 EIA and Abbot HIV-1/2 EIA.

Results: 30/108 (28%) samples were indeterminate and 78/108 (72%) became negative when tested by the HIV-1/2 SIA assay. 17/26 (65%) Dupont HIV-1, 6/13 (46%) Abbot HIV-1 and 7/69 (10%) Abbot HIV-1/2 repeat reactive samples continued to be indeterminate by SIA.

Conclusions: The HIV-1/2 strip immunoblot assay can reduce the number of non-specific p24 reactions when confirming HIV-1 or HIV-1/2 ELISA repeat reactive samples in low risk populations.

POSTER NUMBER 10

Comparison between CHIRON® RIBA™ HIV 1/2 SIA and DBL HIV BLOT 2.2

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Purpose: To compare the performance of RIBA™ HIV-1/HIV-2 SIA (strip immunoblot assay) to DBL HIV BLOT 2.2 Western Blot kit by testing well characterized samples.

Design: Sensitivity was determined by testing the following samples having antibody: 27 HIV-1, 13 HIV-1 seroconversion (SC) from 10 patients, 1 pre-seroconversion (EIA & Western Blot negative, p24 Ag positive), 9 HIV-2, 6 HIV-1/HIV-2 dual infections, and 4 uninfected infants born to HIV-1 infected mothers. Specificity was determined by testing samples from 23 Western Blot indeterminate but uninfected patients. Samples were tested by RIBA™ HIV-1/HIV-2 SIA, DBL HIV BLOT 2.2, Diagnostic Pasteur HIV-2 WB, also Coulter p24 Ag if necessary.

Results: 27/27 HIV-1 positive samples were positive by RIBA™ and DBL. Nine SC samples were from 9 patients, 8/9 were positive by RIBA™ & DBL. 1/9 was indeterminate by both tests. Four SC samples, from one patient, showed RIBA™ positive 5 days before DBL. The pre-SC sample was indeterminate by RIBA™ and negative by DBL. 9/9 HIV-2 were positive by RIBA™ and classified as HIV-2. 6/6 HIV-1/HIV-2 dual infections were positive by RIBA™ and classified as dual; DBL scored 6/6 as positive and classified 6/6 as dual. Of 4 neonatal samples, RIBA™ was positive on 2 and indeterminate on 2; DBL was positive on 1 and indeterminate on 3. 16/23 (69.5%) of Western Blot indeterminate samples became negative by RIBA™.

Conclusion: RIBA™ HIV-1/HIV-2 SIA appears to be more sensitive and more specific than DBL HIV BLOT 2.2 and is able to distinguish between HIV-1 and HIV-2 infections.

POSTER NUMBER 11

SPECIFIC AND SENSITIVE HIV-1/HIV-2 EIA USING *ENV* AND *POL* SYNTHETIC PEPTIDES. N. MONJI, J. MONTANA, C.-A. COLE, M. HIGGS, D. MERCIER, P. SHORES, P. C-D. Su , C.FERRERA, C. JINNEMAN, and P. F. COLEMAN, (Genetic Systems - Sanofi Diagnostics Pasteur, Inc., Redmond, WA 98052)

An improved EIA has been developed which is based on synthetic peptides derived from highly conserved, immunodominant regions of the *env* and *pol* gene products for the detection of antibodies to HIV-1 and HIV-2. The Peptide EIA is an indirect, second antibody sandwich assay in a microplate format. The micro wells are coated with a cocktail of 4 peptides: *env* and *pol* sequences for both HIV-1 and HIV-2, respectively. The assay is performed using the following incubations: Diluted sample - 30 min. (37°C); Working strength HRP Conjugate - 30 min. (37°C); TMB Substrate - 30 min. (RT).

Various serum and plasma samples, including normal donors, commercially available, low-titer sensitivity panels, seroconversion panels and confirmed positives were tested. Of 7634 normal donor samples, 9 were initially reactive (IR = 0.12%) and 4 were repeatedly reactive (RR = 0.05%). The HIV-1/HIV-2 Peptide EIA detected all of the Western blot (WB) positive samples among low titer sensitivity panels. On commercially available seroconversion panels, the HIV-1/HIV-2 Peptide EIA detected samples 1 -2 bleeds earlier than a licensed viral lysate based HIV-1/HIV-2 EIA (124/191 vs. 107/191) and is as sensitive as other licensed EIAs. More than 850 diverse HIV-1 WB positive samples and 300 HIV-2 WB positive samples were all positive (100% sensitivity for both HIV-1 and HIV-2). The sensitivities of the *pol* peptides alone for HIV-1 and HIV-2 were, 97% and 92%, respectively. Twenty one characterized HIV-1 "O" variant samples, tested in France and the US, were all positive by the HIV-1/HIV-2 Peptide EIA.

The HIV-1/HIV-2 Peptide EIA described here is more specific and more sensitive than a viral lysate-based EIA and is well suited for blood screening and diagnostic applications.

POSTER NUMBER 12

Comparison of FDA Licensed Western Blot Assays and Miniaturized Assays for Serum and Dried Bloodspot Confirmation.
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Three licensed Western Blot (WB) kits (Cambridge Biotech HIV-1 Western Blot Kit - Cambridge Biotech, Novapath Immunoblot Assay - Bio-Rad Corporation, Organon Teknika HIV-1 Western Blot Kit - Organon Teknika Corporation) and the miniblot miniaturized WB method for HIV-1 were compared for the ability to confirm the presence of HIV-1 antibodies in serum. US and foreign HIV-1 positive sera (n=142), 11 seroconversion panels (n=84), 1 low and 1 mixed titer panel (n=40) were tested. Dried Bloodspots (DBS) were compared on Cambridge and the miniblot using HIV-1 simulated DBS and 4 DBS seroconversion panels. For the 142 HIV-1 specimens banding patterns for the three licensed kits and the miniblot were essentially equivalent. A reduction in detection of the p55 and p17 bands were observed for the miniblot and Organon Teknika assays. In 8 of 11 seroconversion panels detection of positivity was equivalent in all but 3 panels. The classification of positive in these panels depended on the kit used and did not vary by more than 2 collection periods within a specific panel. Banding patterns for Cambridge and the miniblot for DBS were essentially the same with the miniblot detecting fewer p55 and p17 bands. The miniblot detected antibody 2 collection periods sooner in 2 of the seroconversion panels run. The Cambridge assay required a reduction in the primary incubation to remove background band formation. These data indicate that the 3 licensed kits and the miniblot are equivalent in their ability to detect HIV-1 antibodies in serum. The Cambridge WB assay can be easily modified to detect HIV-1 antibodies in DBS specimens.

POSTER NUMBER 13

COMPARISON OF DRIED BLOOD SPOT (DBS) METHODS USING HIV SEROCONVERSION PANELS AND LOW-REACTIVE SPECIMENS

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AIM: The purpose of this study was to evaluate the sensitivity of DBS techniques using specimens from early HIV seroconversion.

METHODS: DBS spots were prepared by mixing specimens from BBI HIV Seroconversion Panels and Low-Titer HIV Performance Panel with packed red blood cells and dotting on S & S 903 filter paper. Control plasma specimens were prepared by centrifugation of the same blood used for the DBS. DBS were tested by 2 Abbott methods (current and new elution buffers), Genetic Systems, and Organon Teknika HIV EIA assays.

RESULTS: For reactive specimens, the DBS OD readings were lower than the controls by 56%, 14%, 0%, or 37%, respectively for the 4 methods. Some difference was accounted for by greater dilution of DBS specimens than plasma specimens in some methods. The new Abbott elution buffer (3A11E) produced higher reactivity with early HIV seroconversion specimens and lower background OD on nonreactive specimens than the current elution buffer (1A96) or the other 2 methods. The four DBS procedures identified 14, 15, 11, and 8 reactive specimens, respectively, in comparison to the controls with 16, 16, 11, and 12 reactive specimens.

CONCLUSION: Results indicate differences in sensitivity of the DBS methods related to DBS eluate dilution, qualities of the elution buffer, and EIA kit sensitivity.

POSTER NUMBER 14

Stability of DBS Samples Exposed to Heat and Humidity in Three Shipping Envelopes

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Background: The search to expand HIV-1 testing has led to application of dried blood spot (DBS) testing to home collection. Previous data has demonstrated that DBS stored or shipped could deteriorate under humid conditions. This study compared the stability of DBS samples exposed to heat and humidity for changes in EIA reactivity using three shipping envelopes.
Materials & Methods: 40 previously characterized HIV antibody positive and 10 HIV antibody negative serum or plasma samples were reconstituted with HIV negative packed outdated red blood cells to achieve normal physiologic concentrations. The 50 mock blood samples were spotted onto filter paper (Schleicher & Schuel #903) in four 3/8 inch diameter blood spots. Blood spots were air-dried 30 minutes, packaged into foil envelope with desiccant, mail envelope, or mail envelope with desiccant. Remaining DBS samples were stored in an incubator at 37 C at 95% relative humidity. 50 samples from each of the 3 packaging methods were removed prior to exposure, and after 4, 7, 10, and 14 days. Samples were eluted and analyzed at MetPath Clinical Laboratories, Wood Dale, IL, using EIA according to package instructions (Organon Teknika Corp.).
Results: Under conditions of heat and humidity, EIA reactivity in DBS samples was observed to decline over time. An air-impermeable barrier with desiccant protects DBS samples from deterioration when compared to air-permeable envelopes currently recommended by NCCLS. In this study, the deterioration differential was significant at 10 and 14 days ($p < .001$).
Conclusion: EIA reactivity for DBS samples exposed to high heat and humidity is better preserved when shipped/stored in a barrier envelope with desiccant when compared to air-permeable envelope methods.

POSTER NUMBER 15

Dried Blood Spot HIV PCR Improves the Diagnosis of HIV in Infants. Rick Galli, Carol Major, Stanley Read, Perry Gomez, Xiaoyi Li, Rosa Mae Bain, Percy McNeil, Tidiane Sy, George Strunc, M O'Shaughnessy, Sharon Cassol. Ontario Ministry of Health, Hospital for Sick Children, Toronto, British
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The diagnosis of vertically transmitted HIV infection is confounded by the presence of maternal antibodies and requires detection of the virus or viral DNA. We have evaluated recent advances in PCR technology using dried blood spot (dbs) extraction vs whole blood lysis with the Roche Amplicor HIV PCR kit in a prospective study of 178 infants born to HIV infected women in the Bahamas.

Methods: 311 dbs (1.2 cm) were tested for HIV PCR after extraction of hemoglobin using the Amplicor Whole Blood Specimen Preparation Kit(AWBSP), and subsequent recovery of DNA from the filters in 200 ul of a chelex containing solution. 118 whole blood (wb) specimens were prepared using the AWBSP. The PCR testing was carried out using 50 ul of extracted dbs or wb material. Outcome for each infant was determined using PCR and HIV culture, HIV antibody, p24 antigen and long term clinical follow up.

Observations: Of the 77 dbs from 30 infants known to be HIV infected, 74 were PCR positive (96.25%). One infant with an initial negative PCR at 47 days of age (positive on repeat testing), was PCR positive at 19 days and at 3 months. The other 2 negative PCR results were from infants under 2 weeks of age. All 144 dbs from 59 HIV negative infants were PCR negative. The remaining 89 dbs PCR results indicate 5 of the 89 indeterminate babies are HIV infected. All 12 wb specimens from 7 HIV infected infants were PCR positive. The 23 wb specimens from 13 HIV negative infants were PCR negative. The remaining 83 wb PCR results indicate 8 of 44 indeterminate infants are infected. 100% concordance was observed when dbs and wb specimens were examined for the same specimen date (7 positive, 48 negative).

Conclusions: DBS PCR provides a valuable alternative in the diagnosis of vertically transmitted HIV infection. Dbs have advantages in specimen collection, stability, transportation, storage, and the convenience of centralized testing. While PCR may be positive as soon as a few days after birth, optimal performance is after one month of age.

POSTER NUMBER 16

Improved Western Blot Immunoassays for Confirmation of Human Antibody to HIV-1 in Dried Blood Spot Eluates
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An improved version of the Genetic Systems™ Pageblot HIV-1, a "page" format Western blot immunoassay which is performed using either the Genetic Systems (GSC) Integra instrument or the Immunetics Miniblotter apparatus, was developed. The improved assay employs a 1:101 dilution for both serum/plasma and dried blood spot (DBS) samples and the same rapid incubation times as the current product for specimen, conjugate, and color development reagent. Improved reagents include a 5X Specimen Diluent/Wash solution, a working strength Conjugate Reagent containing specific reactivity to IgG, IgM, and IgA, and a working strength Color Development Reagent.

The improved reagents were also configured in a rapid mini-strip format (Genetic Systems HIV-1 Western Blot), which employs the same 1:101 sample dilution and a rapid procedure (60', 45', and 3'). Because of the small strip size, a protocol for confirmation of dried blood spot samples is practicable in this format as well; a single 1/4" DBS punch may be eluted directly in the Western blot reaction tray to yield an effective 1:101 dilution by either a one hour (RT with rotation) or overnight (static) protocol.

Sensitivity for the rapid mini-strip blot was equivalent to a licensed Western blot kit (which uses an overnight procedure) in testing of HIV-1 positive and seroconversion specimens. Equivalent sensitivity and band reactivity for dried blood spot eluates compared to serum or plasma was demonstrated by testing matched serum/DBS pairs. These results support the feasibility of the Western blot immunoassay in either format as a confirmatory method for dried blood spot eluates.

POSTER NUMBER 17

IMMUNE-COMPLEX DISSOCIATION (ICD) OF HIV-1 p24 ANTIGEN AS A FIRST STEP IN THE DETECTION OF PERINATALLY TRANSMITTED HIV-1 INFECTION. BASE DISSOCIATION & ACID ICD COMPARED.

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The transmission rate of HIV-1 from mother to child has been reported to be 10-30% of all births. A reliable means of determining the infected state of a child born to an HIV-1 infected mother is crucial for establishing appropriate therapy. Detection of antibody to HIV-1 proteins is of limited use in these children under the age of 12-15 months because maternal antibodies to HIV-1 are usually present.

Methods that detect direct evidence of virus are more effective in this situation. Nucleic acid amplification and viral culture are often used but are expensive. Direct detection of viral proteins is less expensive but is complicated by the presence of maternal antibodies. ICD detection of p24 antigen, however, can detect p24 antigen in many HIV-1 infected infants.

Fifty-one (51) samples were obtained from pediatric patients, 1-48 months in age, born of HIV-1 infected mothers in Argentina. Twenty-seven (27) of the samples came from children infected with HIV-1 and twenty-four (24) from individuals shown to be non-infected. All samples were tested with the Organon Teknika base and Coulter acid ICD procedures.

Of the 27 samples from infected patients, 19 (70.4%) were reactive with Organon and 15 (55.6%) with Coulter. Of the 24 samples from non-infected patients, 1 was reactive with Organon and 2 were reactive in the Coulter ICD assay. Further testing is being done to confirm reactives and determine antibody loads in these samples.

POSTER NUMBER 18

HIV DRIED BLOOD SPOT (DBS) WESTERN BLOT STUDIES; FEASIBILITY OF USING A STRIP PROCESSOR FOR BOTH ELUTION AND BLOT PROCESSING OF DBS SPECIMENS

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Introduction: Using instrumentation to facilitate running Western blot strips may be a critical adjunct to standardization of this technology. This study was conducted to determine the feasibility of using the programmable SLT Profi-BLOT Western blot strip processor for both elution and processing of DBS eluates.

Experimental: Simulated DBSs were prepared from HIV sera according to the CDC protocol ("Preparation of Dried Blood Spot Materials for Quality Assurance of Assays for Antibodies to Human Immunodeficiency Virus"). The SLT Lab Instruments Profi-BLOT Western blot strip processor was programmed to provide a 60 minute elution of material from DBSs directly into the troughs of the tray. The eluted spots were removed, the Western blot strips added to the eluate in the trough, and a programmed OTC Western blot protocol used to process the strips. Multiple numbers of 1/4" DBSs or 1/8" DBSs from the simulated spots were compared with the serum used to prepare the spots to determine how many punches of spots are necessary to provide results comparable to the serum in an investigational OTC Western blot test system. Actual DBSs from normal adults and neonates were run with the same protocol.

Sensitivity: Comparable Western blot reactivity of the sera could be obtained with their respective simulated DBSs if two (2) spots(1/4") or eight (8) spots(1/8") were analyzed by this method.

Specificity: This procedure did not give abnormal specificity problems with actual normal neonate or adult DBS specimens.

Conclusion: It is feasible to develop instrumentation-assisted methods to detect dilutions of reactive HIV sera without compromising the specificity of testing with normal DBS specimens. Elution of the two 1/4" punched spots directly in the troughs works well; elution of the eight 1/8" punched spots is done better in a test tube with the resulting eluates added to the troughs.

POSTER NUMBER 19

WESTERN BLOT CONFIRMATORY TESTING OF ORASURE ORAL FLUID SPECIMENS.

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Oral fluid specimens collected with the OraSure HIV-1 oral specimen collection device are approved by the U.S. Food and Drug Administration for screening for HIV-1 antibodies with the Oral Fluid Vironostika HIV-1 Microelisa System. A Western blot confirmatory assay has been developed specifically for use with OraSure specimens. The OraSure Western Blot (OWB) uses a 1:8 specimen dilution instead of a 1:50 dilution for serum, specimen incubation time is increased from 1 hour to 3 hours, and a more sensitive enzyme/substrate system is used. Preclinical sensitivity testing revealed that the OWB was positive in 224 of 226 HIV positive individuals. The remaining two were indeterminate with positive gp160 and gp120 bands and indeterminate gp41 and p24 bands. There were no false negatives. Specificity testing demonstrated that the performance of the OWB was very similar to the serum Western blot comparator and showed no false positives. Two hundred forty-five matched serum and OraSure "non-specificity" HIV-negative specimens were also evaluated. The specificity characteristics of the OWB [241 negative or nonviral band only, 4 indeterminate (viral bands)] was superior to those of the serum comparator Western blot [218 negative or nonviral band only, 27 indeterminate (viral bands)]. These results indicate that the OWB represents a promising means of confirming the presence of HIV-1 specific antibodies in OraSure specimens. A large-scale, multi-institutional clinical trial with the OraSure Western Blot is in progress.

POSTER NUMBER 20

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HIV SALIVA TEST FOR SURVEILLANCE AND SURVEYS

While people are reluctant to give blood specimens for HIV testing, they often will not act to reduce HIV infection until they perceive that the virus has come to their community. Saliva is an ideal medium for surveys and surveillance because of its non-invasive nature, ease of collection, and safety for health and laboratory workers. Important to public health agencies are the cost, sensitivity and specificity of the test. As part of our attempt to find a lowcost testing scheme, we evaluated the Omni-SAL® saliva collection device (SDS, Inc.) and the *Detect HIV 1/2* assay (BioChem ImmunoSystems, Inc., Montreal, Canada), using specimens collected in Thailand in the largest comparative study ever done of saliva (AIDS 8, 885-894, 1994). For our blind re-analysis, we selected 986 saliva specimens from two of four sites in Thailand. Known to two of us (RRF and NS) but blind to the laboratory staff, 172 saliva specimens were previously determined to be true HIV+ and 814 to be true HIV- by linked serum ELISA testing and Western blot confirmation.

Saliva specimens were measured with *Detect* up to three times; HIV reaction was defined as OD/COV≥ 1.0 by at least two of the three potential tests. Following these criteria, 168 of 172 HIV+ samples were correctly identified (sensitivity = 97.7%) as were 814 of the 814 HIV- samples (specificity = 100%). Given these findings, if the true HIV prevalence in a tested group was 1.0 %, 5.0% or 10.0%, the saliva test with the excellent specificity and good sensitivity would observe a prevalence of 0.98%, 4.88% or 9.77%, respectively. We feel that the *Detect HIV* 1/2 assay and *Omni-SAL* saliva collection device are ideal for community surveys, follow-up intervention or prevention trials, and surveillance of highly mobile and elusive groups such as drug addicts, homeless persons, or commercial sexworkers. Also to be considered for saliva testing are school or college students, sexually-active single men and women, and pregnant women who might prefer specimen collection in the privacy of the home.

POSTER NUMBER 21

Field Evaluation of Methods to Detect Antibodies to HIV-1 in Oral Fluids. TIMOTHY C. GRANADE[1], SUSAN K. PHILLIPS[1], BHARAT PAREKH[1], PERRY GOMEZ[3], WENDY KITSON-PIGGOTT[2], STEPHANIE LEE-THOMAS[1], AND J. RICHARD GEORGE[1]. [1]Centers for Disease Control and Prevention, Atlanta, GA, [2]Caribbean Epidemiology Centre, Port-of-Spain, Trinidad, and [3]Ministry of Health and Environment, Nassau, Bahamas

Advances in collection technology have resulted in the use of oral fluids (OF) as a clinical specimen for detection of antibodies to HIV-1. Recently, an oral fluid collection device (Orasure) and an enzyme immunoassay (EIA) (Organon Teknika Corp.) have been licensed by the Food and Drug Administration for HIV-1 antibody detection. We collected matched serum and oral fluid pairs (N=4448) from blood donors and patients attending local STD clinics in Trinidad and the Bahamas and tested for the presence of HIV-1 antibodies. Sera were tested by the Abbott HIV AB HIV-1/HIV-2 (rDNA) EIA and positive specimens were confirmed by Cambridge HIV-1 and HIV-2 Western blot (WB). Oral fluid specimens were tested by Murex GACELISA and an OTC research-use-only test and were confirmed by miniaturized Western blot (OFWB). The results of serum EIA and WB determined 475 specimens to be HIV antibody positive. GACELISA detected 472 positive specimens (sensitivity = 99.4%, specificity = 99.4%) and OTC detected 468 positive specimens (sensitivity = 98.3%, specificity = 99.6%). Concordance of OFWB with serum WB was 99.4% and banding patterns determined by both methods were essentially identical. These data further confirm that OF specimens can be tested for HIV antibodies with available tests without significant loss of sensitivity or specificity.

POSTER NUMBER 22

Search for HIV-1 Group O Infections by Peptide Serology

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Since it has been shown that some screening tests for human immunodeficiency virus type-1 (HIV-1) have not been able to detect infections with new highly divergent strains of HIV-1 known as group O, active surveillance for group O infections as well as other variants of HIV-1 will become increasingly important to insure the safety of the blood supply. Most of the reported infections with HIV-1 group O strains have occurred among persons in or from Cameroon or neighboring Gabon. However, the world-wide distribution of these divergent strains is not known. This study uses enzyme immunoassays (EIAs) based on synthetic peptides representing all of the currently known subtypes A-H as well as group O to screen and type 790 sera from the United States and Puerto Rico and 622 African sera. No group O type infections have been found among any of the US/PR samples. Only 2 group O infections were detected among the African sera, both of which were from Cameroon. Although infections with HIV-1 group O strains still appear to be rare, the potential for divergent strains to infect persons living in the U.S. and in some cases to remain undetected by current HIV antibody tests is of concern for the public-health and blood-banking communities. Active surveillance for HIV variants will be important and the evaluation of HIV screening tests for detecting these variants will be critical.

POSTER NUMBER 23

Effects of Sample Preparation, Dilutions and Matrix Addition on the Performance of FDA Approved HIV-1 Tests. B. YEN-LIEBERMAN*, S. SCHINDLER, D. HATCH, A. ROBERTO, L. GADDIS, and R. DOMEN. Cleveland Clinic Foundation, Cleveland, OH.

Samples included in some large scale proficiency testing programs (PT) are processed (pooled, heated, centrifuged, diluted with matrix materials) before packaging. In this study, the effect of sample treatment on the performance of selected FDA approved HIV EIAs was evaluated.

METHODS: The Murex SUDS HIV-1 Test (SUDS), the Cambridge Biotech Recombigen (env & gag) HIV-1 EIA (CBC) and the Abbott HIVAB HIV-1/HIV-2 (rDNA) EIA (HIVAB) were performed according to manufacturer's guidelines. Sample processing was performed at the Cleveland Clinic Foundation according to at least one PT provider. Seroconversion samples were purchased from BBI.

RESULTS: SUDS exhibited 100% sensitivity in comparison with CBC and HIVAB when tested with HIV-1(+) sera. With BBI seroconversion panels, SUDS exhibited 100% correlation with Western Blot. Heating samples at 60°C for 60 min increased absorbance readings with CBC EIA at least 10-fold and gave false positive results with SUDS. The HIVAB test did not give expected results with a diluted HIV-1(+) serum panel. CBC and Murex EIAs were non-reactive with sera diluted to endpoints in pooled negative plasma.

CONCLUSIONS: These data show that there are differences in the performance in HIV tests with PT samples. Diluting in pooled negative plasma, heating and centrifuging may adversely affect the results of some FDA approved HIV tests. Testing processed PT samples may provide an inaccurate assessment of test performance.

POSTER NUMBER 24

An Improved Enzyme Immunoassay for Detection of Human Antibody to HIV-1 Utilizing Viral Lysate and a Purified Recombinant Envelope Antigen.

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An enhanced version of the Genetic Systems™ LAV EIA (GSC LAV EIA) was developed by combining a highly purified recombinant protein, containing antigenic regions of HIV-1 gp120 and gp41, with HIV-1 viral lysate as the capture elements of the solid phase. Addition of the recombinant antigen permitted a reduction in certain viral lysate antigens, e.g. p18, which are a source of false positive reactivity in low prevalence populations, while maintaining high levels of those proteins needed to ensure cross-reactivity with variant HIV subtypes. The assay employs a 1:101 dilution for serum and plasma and a slightly modified protocol with a 1:401 dilution for dried blood spot testing. The resulting assay configuration demonstrated both significant reduction in the detection of indeterminate specimens and significant improvement in sensitivity as measured by detection of early seroconversion specimens when compared to the current GSC LAV EIA.

Banked specimens, selected to be reactive by GSC LAV EIA and indeterminate by licensed Western Blot, were tested with the improved assay configuration. Of 100 indeterminate samples reactive by GSC LAV EIA, only 23 (23%) were reactive with the improved assay. In testing of 12 commercial seroconversion panels, the improved assay configuration detected seroconversion 0-3 bleeds earlier than the current GSC LAV EIA. The improved assay cross-reacted with 90% (9 of 10) of the HIV-2 samples tested, which was equivalent to the current LAV EIA. Banked specimens from a plasma center were tested with the improved assay to estimate specificity in a normal donor population: of 1000 specimens tested, one was initially reactive (IR = 0.1%) and none repeated (RR = 0.0%). Dried blood spot testing with the improved assay utilized either a 1 hour (RT with shaking) or overnight (2-8°C static) elution protocol. The performance for dried blood spot eluates by the modified protocol was equivalent to the 1:101 dilution of serum or plasma.

POSTER NUMBER 25

Performance of Laboratories Testing HIV-1 Seroconversion Samples in the CDC Model Performance Evaluation Program August 1994 Survey Panels

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The CDC Model Performance Evaluation Program (MPEP) evaluates the quality of performance of laboratories testing for HIV-1 antibody to assist them in identifying and correcting problems so that testing performance will be maintained or improved. As a part of the performance evaluation process, the testing of HIV-1 antibody-weak positive samples was assessed by sending panels that contained HIV-1 seroconversion samples to participating laboratories in August 1994. Each laboratory received 2 HIV-1 seroconversion samples among the 6 samples in their panel. The HIV-1 antibody (Ab) reactivity of donor samples in this survey was determined first by CDC after testing samples with all HIV-1 enzyme immunoassay (EIA) and Western blot (WB) kits licensed by the Food and Drug Administration (FDA) and interpreting WB results using the ASTPHLD/CDC HIV-1 WB interpretive criteria. Of 1530 final EIA interpretations for the HIV-1 seroconversion samples tested by laboratories using FDA-licensed EIA kits, 118 (7.7%) EIA negative interpretations were reported. Among 376 Western blot interpretations for the HIV-1 seroconversion samples tested by laboratories using FDA-licensed WB kits, 195 (51.9%) indeterminate, 168 (44.7%) reactive, and 13 (3.5%) nonreactive interpretations were reported. Of 62 IIF interpretations for these same samples tested by laboratories using an FDA-licensed HIV-1 IFA kit, 27 (43.5%) reactive, 7 (11.3%) indeterminate, and 28 (45.2%) nonreactive interpretations were reported. Of 30 interpretations for these samples tested by laboratories using an FDA-licensed rapid qualitative microfiltration EIA 18 (60%) reactive, 11 (36.7%) nonreactive, and 1 (3.3%) indeterminate interpretations were reported. Final EIA and WB test interpretations varied for some HIV-1 seroconversion samples depending on which FDA-licensed EIA or WB test kit was used by the laboratory.

POSTER NUMBER 26

DETECTION OF ERROR IN INFECTIOUS DISEASE TESTING: KIT VALIDATION CRITERIA VERSUS INDEPENDENT CONTROL VALUES

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AIM: Our goal was to compare independent low-reactive controls and manufacturers' run validation criteria as indicators of systematic errors in infectious disease assays.

METHODS: Testing problems such as kit deterioration, faulty pipettors, and inadequate incubation conditions were simulated by decreasing reagent concentration, sample volume, incubation temperatures, and incubation times. Manufacturers' positive and negative kit controls and external controls were tested under normal and simulated error conditions. Infectious disease EIA tests that were studied included: HIV, HTLV, HBsAg, HBc, and HCV.

RESULTS: Under simulated error conditions in 32 runs, the kit positive control indicated only 4 invalid runs (13%); the kit negative controls indicated 3 invalid runs (9%). The external controls warned of a problem in 20 runs (63%) that were outside the 3sd range and 24 runs (75%) that were outside the 2 sd range. The external negative control provided a warning in 9 (28%) or 13 (42%), for the 3sd and 2sd ranges, respectively.

CONCLUSION: We conclude that monitoring of external run controls using 2sd and 3sd rules is more useful for identifying systematic errors or technical problems than the sole use of the manufacturer's acceptance ranges for the kit calibrators.

POSTER NUMBER 27

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EVALUATION OF HIV-1 TEST STRIPS

In an effort to simplify testing for HIV antibodies in serum and plasma, we have developed test strips that are as easy to use as contemporary pregnancy tests. A total of 3281 serum specimens from four different geographic locations (Mexico, Thailand, USA, and Myanmar) have been analyzed with these strips, Sero•Strip HIV, in five blind studies and by personnel from different laboratories. All specimens were also analyzed with at least one EIA and if discrepant results were obtained, further analysis with a reference method was performed (either Western blot or LiaTek). The clinical status of patients with AIDS was also considered for the classification of specimens. One of the specimens provided indeterminate results with the reference method and was excluded for calculations of diagnostic sensitivity and specificity of the Sero•Strip tests (Table 1).

One of the false negative specimens was also non-reactive with the reference method but the patient was in an advanced stage of AIDS and under AZT treatment (Study 2). It can be reasonably assumed that the immune system of this patient was compromised (no circulating immunoglobulins). Another false negative specimen originated from a deteriorated sample (Study 5). The third false negative specimen was repeatedly reactive on the Sero•Strip HIV assay upon re-analysis (Study 1).

	Total	Sero•Strip pos.	neg.
Positive	761	758	3[1]
Negative	2519	1	2518
Indeterminate	1	0	1
Sensitivity		99.6%	
Specificity		99.96%	

Table 1. Diagnostic sensitivity and specificity of the Sero•Strip HIV-1 test with specimens collected at four different geographic locations.

[1] One specimen was also negative with the reference method; patient at advanced stage of AIDS and under AZT treatment (i.e., break-down of the immune system). Excluding this specimen, the sensitivity would be 99.7%.

So far, we find that other (non-HIV) diseases or medical conditions (a total of 403 specimens) do not affect the specificity of the Sero•Strip test. The sensitivity of the Sero•Strip HIV was comparable to traditional enzyme immunoassays; also, the specificity exceeded the results obtained with the enzyme immunoassays used in the respective studies.

POSTER NUMBER 28

RESULTS OF A TELEPHONE SURVEY OF LOCAL HEALTH DEPARTMENTS ON LABORATORY USE FOR THE HIV-1-INFECTED PATIENT

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During September through November 1994, the Laboratory Assurance Program at San Diego State University performed a national telephone survey of local health departments. Out of 361 attempted surveys, 89% (320) were completed, 4% declined to participate, 6% were considered ineligible to respond as they were boards of health making policy decisions only, and 1% were nonparticipants. Most of these local health departments had HIV testing and counseling programs (80%), fewer (28%) had an HIV early intervention program. By far, the majority of departments performing testing used the HIV-1 EIA screen (94%) and Western blot test (91%) in the evaluation and treatment of clients in their programs. Yet, less than half (48%) had their own laboratory. Most of the laboratories performing these tests were rated good to excellent. Other testing results used by these departments were CD4+ T-cell (49%), IFA (19%), HIV-2 EIA screen (19%), and PCR (11%). The laboratories utilizing CD4+ T-cell testing results send most of this work to commercial or state laboratories.

POSTER NUMBER 29

Blind Performance Evaluation: Achieving A More Accurate Measure of HIV-Antibody Testing Performance

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Using federally funded counseling and testing (CT) sites, the CDC Model Performance Evaluation Program (MPEP) implemented a blind performance evaluation study to assess the performance of HIV-antibody testing laboratories. A total of 162 CT sites located in 44 states and 99 corresponding testing laboratories are participating in this study. Split (duplicate) specimens were collected from patients who were determined to be at high risk for HIV infection to increase the probability of collecting HIV-antibody-positive specimens in addition to the HIV-antibody-negative specimens. One of the duplicate (blind) specimens was sent to the testing laboratory through the CT site routine requisition procedures, while the other duplicate specimen was forwarded to CDC, processed, and sent as a known specimen to the same testing laboratory that had received the original patient specimen. Among the testing results reported for the 4,704 HIV-antibody-positive and antibody-negative specimens collected to date, 19 errors were recorded. An error was recorded if the screening or supplemental test result was not consistent among the duplicate (split) specimens from the same patient. Discrepancies in both screening and supplemental testing for the open and blind specimens showed a 0.15% error in the open specimen testing and a 0.25% error in the blind specimen testing. Preliminary results from both phases of this study indicate that these discrepancies are random and not repetitive over time.

POSTER NUMBER 30

DESIGN OF RFP PRODUCT EVALUATION: HIV-1 ANTIBODY EIA SYSTEMS

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ABSTRACT

The City of New York's Department of Health performs approximately 500,000 HIV-1 antibody screening EIA's each year. Therefore, it is essential that this laboratory periodically re-evaluates all available FDA licensed HIV-1 antibody EIA systems, on a request for proposal (RFP) basis, for the issuance of multi-year testing system(s) requirement contracts. In November of 1993 the Department of Health advertised for proposals from vendors of HIV-1 antibody EIA systems who were interested in having their products evaluated by the City's HIV testing laboratory. Interested vendors responded with written proposals in accordance with stated City guidelines. Vendors, successful in their written response, were invited to have their products evaluated by the HIV testing laboratory. The basic design of the evaluation was explained to each of the invited vendors prior to the delivery of kits and equipment. Each vendor was allowed to perform up to 60% of the actual specimen testing. The evaluation was designed to determine the following from each vendor's HIV-1 antibody screening EIA kit/system: (A) sensitivity, (B) specificity, (C) reproducibility, (D) productivity, and (E) LAN compatibility. The initial design for specimen selection consisted of 1000 to 1200 random fresh serum submissions and 500 previously characterized repository specimens (from approximately 150 known HIV-1 antibody positive patients, 50 known negatives and the remainder from various combinations of HIV-1 EIA and WB inconclusives), all with available patient history. The outline of the specifics of our initial evaluation design and how this design will be modified for future application are presented.

POSTER NUMBER 31

VALIDATION OF AN AUTOMATED MICROPLATE PROCESSOR, T. GROVE; C. JINNEMAN ; K. SHRIVER, Genetic Systems/Sanofi Diagnostics Pasteur, Seattle, WA; R. CREAGER, Sanofi Diagnostics Pasteur, Chaska , MN

The Procession™ Microplate Processor automatically incubates, washes, adds reagents, and reads the optical densities (O.D.s) of microplate based EIAs.

We performed a comparison study of the Procession™ with a reference method using the Genetic Systems/Sanofi Diagnostics Pasteur HIV1/HIV2 EIA , LAV, ALT and HBsAg EIAs. The purpose was to demonstrate equivalent results with an accepted reference method. The reference method consisted of : Pipetman® pipets for preparing sample dilutions and reagents, a Biotek® EL403 washer, a Titertek® 8-channel pipet for dispensing reagent, and a Biotek® EL312 reader.

We tested and compared a variety of assay parameters against the standard reference method: sensitivity, specificity, precision, carryover, reagent stability, and method correlation. In addition, for the LAV assay we tested dried blood spots eluates.

The results are as follows:

	LAV	HIV-1/HIV-2	HBsAg	ALT
Specificity of Normal Donors (Mean S/C ; N)	0.145 / 300	0.129 / 290	0.338 / 300	N/A
Sensitivity	Equivalent to Reference	Equivalent to Reference	< 0.29 ng/ml ad < 0.34 ng/ml ay	N/A
Correlation to Reference Method (R)	> 0.95	> 0.95	> 0.95	> 0.95
Precision (%CV)	<10 @ 1.786 <30 @ 0.055	<10 @ 1.104 <30 @ 0.033	<10 @ 1.415 <30 @ 0.037	<20 @ 17 IU/L <10 @ 38 IU/L
Carryover	None	None	None	N/A
Reagent Stability	Equivalent to Reference	Equivalent to Reference	Equivalent to Reference	Equivalent to Reference
Dried Blood Spot Specificity (Mean S/C; N)	0.412 / 85	N/A	N/A	N/A

Our results indicate that the Procession™ Microplate Processor provides assay results which are equivalent to the reference method results. The Procession™ will be an important tool for the automation of microplate EIAs.

POSTER NUMBER 32

THE EFFECT OF MICROBIAL CONTAMINATION ON HIV ANTIBODY DETECTION

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Background: A number of HIV antibody positive repository specimens have given discrepant results between current HIV antibody assays when tested after storage. These specimens have also been found to be microbially contaminated. The following is an investigation to determine if microbial contamination is responsible for the discrepant results and to investigate the mechanism of the signal suppression.

Methods: I) HIV repository specimens, which were microbially contaminated, were tested by EIA, Western Blot (WB) and Immunoflourescence (IFA) assays. II) One HIV antibody negative and six HIV antibody positive specimens were experimentally inoculated with bacteria and evaluated for performance in the Abbott HIV-1/2 rDNA EIA (LN 3A77). III) Samples were evaluated by HPLC sizing for IgG integrity and its relationship to EIA detectability. HPLC fractions were evaluated for total IgG and HIV specific IgG using Goat Anti-Human and HIV antigen HRPO conjugates.

Results: I) Bacterially contaminated repository specimens showed discrepant results between historical and current testing, as well as discrepancies among current EIA and WB assays. II) Direct inoculation of microbes did not immediately impact HIV antibody detection, but HIV antibody detection decreased after 3 months of storage. One specimen was no longer EIA positive after 3 months. III) The microbially contaminated samples showed no IgG peak at 160,000 MW. IgG activity was detected in fractions at less than 20,000 MW, indicating structural degradation. No IgG reactivity was detected using the HIV antigen conjugate in these degraded IgG fractions. Minimal HIV reactivity was observed with the Goat Anti-human conjugate within these fractions.

Conclusion: Microbial contamination of human specimens has a significant impact on HIV detectability. Data suggests that degradation of IgG in such samples is the likely cause of the compromised detection.

POSTER NUMBER 33

SIGNIFICANCE OF HIV-2 SCREENING IN GEOGRAPHIC AREAS WITH HIGH INCIDENCE OF HIV-1. ROMULO MORALES, MAHIN PARK, RAKSHA MODI, and CHARLES SCHABLE*. Georgia Public Health Lab and the Centers for Disease Control*. Atlanta, GA.

Although HIV-2 infections were first reported exclusively in West Africa, in spite of its low prevalence, so far, this infection has been reported in several continents including North America. Atlanta is becoming a center of international travel and its African American population for Metropolitan Statistical Area constitutes 28.5% of the total population. The purpose of this study was to determine the prevalence of HIV-2 infections in a total of 127,790 specimens we had already screened for HIV-1 infection during 1994. For this study, we chose 588 specimens which were either negative (43%) or indeterminate (54%) by our Western Blot analysis. In addition, we included 1.5% Western Blot positive specimens for control. When tested by ELISA for HIV-2, 10% turned out positive in the first and 7% in the repeated screening assay. When tested against the synthetic peptide assay for HIV-2 (test performed at the CDC), 1% (7 specimens) turned out positive for HIV-2. Dot Blot assay at the CDC demonstrated that 3 specimens were positive for both HIV-1 and 2. Further testing is underway to determine if any of these specimens are HIV-2 positive. The result of our study showed that some of HIV-1 positive specimens demonstrated cross reactivity with HIV-2 in the ELISA. In spite of this cross reactivity, we think it is important to test all repeat reactive HIV-1 specimens by HIV-2, as well. We speculate that some HIV-1 infected individuals, especially those in the high risk category, may be dually infected with both viruses. Additionally, by testing HIV-1 alone, we may miss very few HIV-2 positive patients. Although confirmation of HIV-2 infection is only investigational, it can provide important epidemiological information for prevention of this deadly disease.

POSTER NUMBER 34

HIV-2 POSITIVE BLOOD DONOR IN DELAWARE.

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Blood Bank of Delaware; Newark, DE,[1] Centers for Disease Control and Prevention, Atlanta, GA[2] and Delaware Public Health Laboratory, Smyrna, DE 19977[3] and Genetic Systems/Sanofi Diagnostics Pasteur, Redmond, WA[4]

A 30 year old black female, who is a U.S. citizen born in the United States, presented at the Blood Bank of Delaware as a first time blood donor in June 1994. The donor initially denied all risk factors at the time of donation. Initial and repeat testing of the blood donor sample was performed with the Genetic Systems™ HIV-1/HIV-2 EIA and found to be repeatedly reactive. The HIV-1 Western Blot was positive when tested at DPHL. An investigational HIV-2 Western Blot (Cambridge Bioscience) was found to be strongly positive. Testing at CDC with the investigational Genetic Systems GENIE™ HIV-1/HIV-2 rapid test produced 4+ reaction for HIV-2 and a 1+ reaction for HIV-1. The investigational Biochem Immuno Systems peptide EIA was positive for HIV-2 and weakly positive for HIV-1. The HIV-1 Western Blot was indeterminate at CDC and an investigational HIV-2 Western Blot testing was positive. In addition, at CDC, the Waldheim Pharmazeutika (Austria) Fluorognost™ HIV-1 IFA was positive and the investigational Fluorognost™ HIV-2 IFA was strongly positive. This first time blood donor is the first known HIV-2 positive donor detected since the institution of combination HIV-1/HIV-2 antibody testing in blood banks in 1991 and it is the second known HIV-2 positive blood donor found in the United States.

POSTER NUMBER 35

FALSE-NEGATIVE FLUOROGNOST HIV-1 IFA RESULTS

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On February 5, 1992, the FDA approved "Fluorognost HIV-1, IFA" as an indirect immunofluorescence assay for both a confirmatory and screening assay for the detection of antibodies to HIV-1 in human serum or plasma. Since May of 1986 the Los Angeles County Public Health Laboratory has used the IFA assay as a supplementary test in conjunction with the HIV-1 Western Blot for confirmatory testing. Originally, the Public Health Laboratory used the IFA slides prepared by the Viral & Rickettsial Disease Laboratory of the California State Department of Health Services (CSDH) for testing but converted to the Fluorognost product after it was FDA approved. At this time the microbiologists performing the tests successfully completed the proficiency test provided by Fluorognost.

Over a seven month period in 1994 we have documented 28 cases in which the EIA was repeatedly reactive, the Western Blot was reactive but the Fluorognost IFA was negative or nonspecific. Because of the discrepant readings, some of these sera were sent to the CSDH for repeat testing. Their testing verified our results.

Our conclusion is that, in our laboratory, the Fluorognost procedure is not as sensitive as the Western Blot in detecting HIV-1 antibodies. Specimens giving EIA repeatedly reactive and IFA negative results should always be followed up by a Western Blot.

POSTER NUMBER 36

URINE HIV1 ANTIBODY TESTING TO DETERMINE PREVALENCE IN ONTARIO CORRECTIONAL FACILITIES.

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Objective: To determine rates of HIV1 infection in those entering jails, detention and youth centres. To compare two methods of detecting HIV1 antibody in urine specimens.

Methods: Leftover urine specimens routinely obtained from adults and young offenders admitted to 42 Ontario facilities between February and August 1993, were tested in an unlinked, anonymous prevalence study. Data on gender, age and history of injection drug use (IDU) was collected. Urines were screened using a modified HIV1 EIA kit (Cambridge Biotech Recombigen) and confirmed (after 10x concentration) for HIV1 antibody with a modified in house western blot (WB) assay. In addition, all reactive, grey zone and 5400 randomly selected negative specimens were tested using the Murex GACELISA HIV1/2 kit. Additional testing (WB, GACPAT, RIPA) was carried out on specimens giving discrepant results.

Results: Urine specimens were tested for 87.9% of new entrants. Among adult males (n=9201), 0.99% were HIV positive; among adult females (n=1302), 1.23% were HIV positive. For those with a history of IDU, 3.6% of males were positive, as were 4.2% of females. None of the young offenders were HIV positive. 106 urine specimens contained HIV antibody. All 106 were detected by the GACELISA assay, while the CBC assay detected 95/106 (89.6%). The respective specificities of the two assay were: CBC 99.98% (5528/5529) and GACELISA 98.6% (5450/5529) Additional testing of discrepant specimens confirmed western blot results.

Conclusions: For the first time, Canadian officials have an estimate of the number of HIV infected individuals admitted to Ontario prisons. A strategy for dealing with HIV is needed. Urine testing provides accurate estimates of HIV prevalence. A dual ELISA algorithm for determining HIV prevalence using urine specimens can provide a convenient, cost effective and accurate estimation of HIV infection in various populations.

POSTER NUMBER 37

COMBINATION OF LINE ASSAY (LIA) AND p24 ANTIGEN EIA IMPROVES CONFIRMATION OF HIV-1 INFECTION AT THE EARLY STAGES OF SEROCONVERSION.

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Specimens and Methods: Twenty four specimens obtained at the early stages of seroconversion from 9 subjects were evaluated. All were reactive by HIV-1/2 3rd generation EIA (Abbott) but either negative (n=6) or indeterminate (n=18) by conventional Western blot (Cambridge/Biotech). They were tested by LIA and p24 antigen EIA (Abbott). Identical interpretation criteria (CDC) were used for LIA and Western blot.

Results: Using Western Blot, 10/18 (55%) indeterminate and 5/6 (83%) negative samples were positive for p24 antigen. Using LIA, 15/24 (62%) samples were classified as positive, only 4 (17%) were indeterminate and 5 (21%) were negative. Of the 4 LIA-indeterminate samples, 2 were positive for p24 antigen and 2 had anti-gp41 antibodies. Four of the five LIA-negative samples were positive for p24 antigen. The HIV-1/2 EIA was positive a mean 4.1 days before LIA and 9.8 days before Western blot.

Conclusion: The combination of LIA and p24 antigen testing greatly reduces the number of seroindeterminate results for samples obtained at the early stages of seroconversion.

POSTER NUMBER 38

Hepatitis B and C as Markers of HIV Risk among Injecting Drug Users (IDU), Newark, NJ and San Francisco (SF), CA. H. WEINSTOCK, C. SPRUILL, G. LEMP, R. ALTMAN, W. PIZZUTI, A. BACK, R. PREVOTS, M. GWINN. CDC, Atlanta, GA; SF Dept of Public Health, SF, CA; and NJ State Dept of Health, Trenton, NJ.

Like HIV, hepatitis B and C are transmitted parenterally. Using hepatitis B and C as markers of HIV risk behavior in IDU with different HIV prevalences, we conducted unlinked surveys of HIV antibody, hepatitis C antibody (anti-HCV), and hepatitis B core antibody (anti-HBc) among clients entering drug treatment centers (DTC) in Newark, NJ (n = 245) and SF, CA (n = 289) in 1990 and 1991. HIV, anti-HCV, and anti-HBc prevalence increased with age in both DTC. In Newark, HIV seroprevalence increased from 9% in the 20-24 age group to 39% in the 40 + age group. Anti-HCV increased from 30% to 77% and anti-HBc increased from 15% to 74%. In SF, seroprevalence also increased with age, from 4% among 20-24 year olds to 8% among those $\geq$ 40 years for HIV, from 56% to 92% for anti-HCV, and from 33% to 79% for anti-HBc. Age-adjusted seroprevalence rates were as follows:

DTC	HIV	Anti-HCV	Anti-HBc
Newark	29%	63%	56%
San Francisco	6%	79%	60%

Similar rates in both centers for anti-HCV and anti-HBc suggest a similar prevalence of risk behaviors for parenteral HIV transmission. The lower HIV seroprevalence in SF may be due to other factors, such as social mixing patterns among IDU that result in a lower probability of contact with HIV-infected persons. Nevertheless, these data suggest that IDU in SF may be at high risk for HIV.

POSTER NUMBER 39

Can a single HTLV confirmatory assay give an accurate result?

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We evaluated 2460 HTLV-I EIA repeat reactive samples between April and December of 1994 using the *Diagnostic Biotechnology HTLV Blot 2.3* (DB WB) and HTLV-I RIPA for confirmation of antibodies to HTLV-I and HTLV-II. 84% of these samples were from blood banks and the remainder were for diagnostic purposes. A sample is identified as anti-HTLV positive with reactivity to *gag* (p19/p24) and *env* as rgp21 (DB WB) and gp61 (RIPA). If a sample is also reactive to rgp46I or rgp46II by DB WB, then it is ultimately classified as anti-HTLV-I or anti-HTLV-II, respectively. With this algorithm, *env* detection by the DB WB must be substantiated with reactivity to gp61 by RIPA.

Analysis of this population indicated 21% positive, 54% inconclusive and 25% negative. Of the positive samples, there were 67 anti-HTLV positive, 165 anti-HTLV-I and 278 anti-HTLV-II. Among anti-HTLV positives, *gag* predominated as p24 in nearly 70% of samples, whereas 30% had p19 and p24. No sample was classified as anti-HTLV positive with the pattern of p19, rgp21, and gp61. Of the majority of the inconclusive samples, 71% were p19/p24, 9% were rgp21 only, and 12% were p19/p24 and rgp21.

We further reevaluated these results using only the DB WB interpretation criteria. Those samples reactive to p19/p24 and rgp21 would now be classified as anti-HTLV positive. An additional 158 samples were then classified as anti-HTLV positive. Of these samples, 84 were p19 and rgp21, 63 were p24 and rgp21, and 11 were p19, p24, and rgp21. Additionally, there were 5 samples which were *gag* plus *env* as rgp21 and rgp46I/II in which no gp61 was detected and additional testing could not identify *env*. This redistribution results in 27% positive, 48% indeterminate and no change for the negative samples of this population.

Our evaluation shows that based on the DB WB interpretation criteria alone, an additional 6% of this population would be identified as anti-HTLV positive. Reactivity to gp61 was detected in 99% of samples with *gag* and 2 *env* (rgp21 and rgp46I/II), whereas reactivity to gp61 was detected in 29% of samples with *gag* (p19/p24) and a single *env* (rgp21) by DB WB. We conclude that using the DB Western blot alone when *gag* and *env* (rgp21 only) reactivity is present, without detection of envelope by an alternate method, is an inaccurate means to assess the presence of antibodies to HTLV and results in falsely positive sample classification. Therefore, we use the HTLV-I RIPA as an obligatory partner to the DB Western blot.

POSTER NUMBER 40

Comparison of Enzyme Immunoassays and Supplemental Tests for anti-HTLV-II.

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The purpose of this study was to evaluate the sensitivity of supplemental tests for detection of anti-HTLV-II samples. A 40 member panel was used for evaluation consisting of 35 HTLV-II samples (18 undiluted and 17 serially diluted from 4 samples), 3 HTLV-I samples (1 undiluted and 2 diluted), 1 indeterminate sample, and 1 negative sample. The panel was used to compare the HTLV-II sensitivity of 4 HTLV screening assays (HTLV-II viral lysate EIA, HTLV-I/HTLV-II viral lysate EIA, and 2 HTLV-I viral lysate EIAs) with 7 supplemental tests (HTLV-I/II western blot, HTLV-II viral lysate western blot, 2 HTLV-I RIPAs, HTLV-I IFA, HTLV-II IFA, and HTLV-II RIPA).

The 4 enzyme immunoassays detected 33/35, 28/35, 18/33, 18/35 of the HTLV-II samples, respectively. Using the U.S. Public Health Service criteria, the HTLV-I/II western blot and the HTLV-II western blot detected 16/35 and 7/35 of the HTLV-II samples, respectively. The HTLV-I RIPA which used a gp61 env band was reactive with 13/35 and the HTLV-I RIPA which used a gp68 env band reacted with 11/35 HTLV-II samples. The HTLV-I IFA detected 13/35 and the HTLV-II IFA detected 20/35 HTLV-II samples. The HTLV-II RIPA which used a gp67 env band reacted with 12/35 HTLV-II samples. The HTLV-I samples were detected by all EIAs and supplemental tests with the exception of the HTLV-II western blot and the HTLV-II RIPA. The indeterminate specimen was reactive only on the western blots and the negative sample was negative by all EIAs and supplemental tests.

These data clearly demonstrate the enhanced sensitivity of the HTLV-II and HTLV-I/HTLV-II enzyme immunoassays for these HTLV-II samples when compared to the 7 supplemental tests.

POSTER NUMBER 41

A Consensus PCR Primer/Probe Set Capable of Detecting Known HIV-1 Subtypes.

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The availability of sequence information from a large number of HIV-1 isolates has permitted the classification of various viral sub-types. Of particular interest, a divergent strain of HIV-1 (MVP5180), now characterized as subtype O, has been isolated from patients in Cameroon in West-Central Africa. Although the genomic organization of the subtype O isolate is similar to other HIV-1 subtypes, the viral sequence varies significantly. We have tested a primer/probe set targeting a conserved region of the HIV-1 viral genome for its ability to amplify all known subtypes. By amplifying under optimal conditions, using lower stringency annealing conditions, and designing primers ≥ 28 base pairs in length and terminating with a 3' thymidine, the probability of detecting all known subtypes should be enhanced.

Representative isolates of subtypes A-F and O were successfully amplified and detected with the consensus primer/probe set. In addition, we have also analyzed 132 HIV-1 seropositive specimens from the Cote d'Ivoire by PCR for the presence of HIV-1 DNA. One hundred-thirty (98.6%) were PCR positive, and two (1.4%) PCR negative. The two PCR negative samples were negative with multiple primer sets. Reasons for the inability to amplify these samples by PCR may be viral load, specimen quality, or more divergent strains not yet identified.

POSTER NUMBER 42

The Use of Quantitative PCR to Monitor HIV Viral Load

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A quantitative RNA PCR assay that has the ability to discern three-fold differences with 87% power has been used to measure HIV viral load in a broad spectrum of patients. With an analytical sensitivity of 200 copies/ml, quantification of HIV RNA in individuals with CD4 counts and in those with undetectable p24 antigen has been achieved. Because as little as 50 µl of plasma/sera is required, quantification of HIV in pediatric patients is feasible.

The assay has been used to monitor various antiretroviral therapies. A sharp decline in viral RNA load is observed after initiation of successful intervention followed by a subsequent rise in viral load. The increase in viral load during therapy appears to be associated with the emergence of antiviral resistant mutants. Where p24 antigen is measurable, the quantitative RNA results correlate with antigen titre. This assay will permit expanded entry into clinical trials, allow measurement of viral load throughout therapy, and enable balancing the benefits of therapy with the deleterious side effects of intervention strategies.

The quantitative assay used offers numerous advantages over conventional RNA quantitative assays: (1) the RNA extraction procedure avoids ultracentrifugation and use of organic solvents; (2) reverse transcription and amplification is carried out with a single enzyme in a single buffer solution; (3) dUTP and UNG are incorporated to ensure that previously amplified material does not contribute to the signal; (4) an internal quantitation standard is used to monitor reaction variability; and (5) quantitative detection is achieved with a colorimetric microwell format. The assay has a four-log dynamic range and can be completed within 8 hours. (*J. Clin. Microbiol.*: Feb. 1994).

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